

ALLOZYME EVIDENCE FOR THE HYBRID ORIGIN OF
DESMODIUM HUMIFUSUM (FABACEAE)

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ABSTRACT. *Desmodium humifusum*, one of the rarest members of the New England flora, always occurs with two conspecifics, *D. paniculatum* and *D. rotundifolium*, and a hybrid origin for *D. humifusum* has been proposed. Protein (allozyme) electrophoresis was used to test this hypothesis. Allozyme data demonstrated that the extant *D. humifusum* populations totaled eight genetic individuals rather than the 100+ previously estimated. The Rogers genetic similarity between the putative parental species was 0.797 and they were fixed for different alleles at a single locus, *Tpi-1*. All but one individual of *D. humifusum* were heterozygous at this locus, combining alleles unique to both of the putative parental species. *Desmodium humifusum* exhibited excess heterozygosity (relative to Hardy-Weinberg expectations), in sharp contrast to the consistent heterozygote deficiency in the parental species. *Desmodium humifusum* consists of both F₁ interspecific hybrids, as well as later-generation hybrids; introgression between the parental species was not obvious.

Key Words: *Desmodium humifusum*, *D. paniculatum*, *D. rotundifolium*, hybridization, allozymes, rare species, New England flora

Ground-spreading Tick-trefoil, *Desmodium humifusum* (Muhl.) L. C. Beck (Fabaceae) is a rare and enigmatic member of the New England flora. Its obscurity owes not only to its rarity, but also to the general difficulty of species delimitation in this genus. Additional confusion has resulted because the name of a related species, *D. glabellum* (Michx.) Alph. de Candolle [= *Meibomia glabella* (Michx.) Kuntze], was misapplied to this species (Gleason and Cronquist 1963; Robinson and Fernald 1908; Vail 1892). The nomenclatural error was subsequently corrected (Gleason and Cronquist 1991; Schubert 1950a) and a detailed description of *D. humifusum* was provided by Schubert (1950b).

Prior to 1996, *Desmodium humifusum* was listed as a “Category 2” species by the U.S.D.A. Fish and Wildlife Service [Federal Register 58(188): 51144]. The Category 2 list comprises species under consideration for protected status but for which avail-

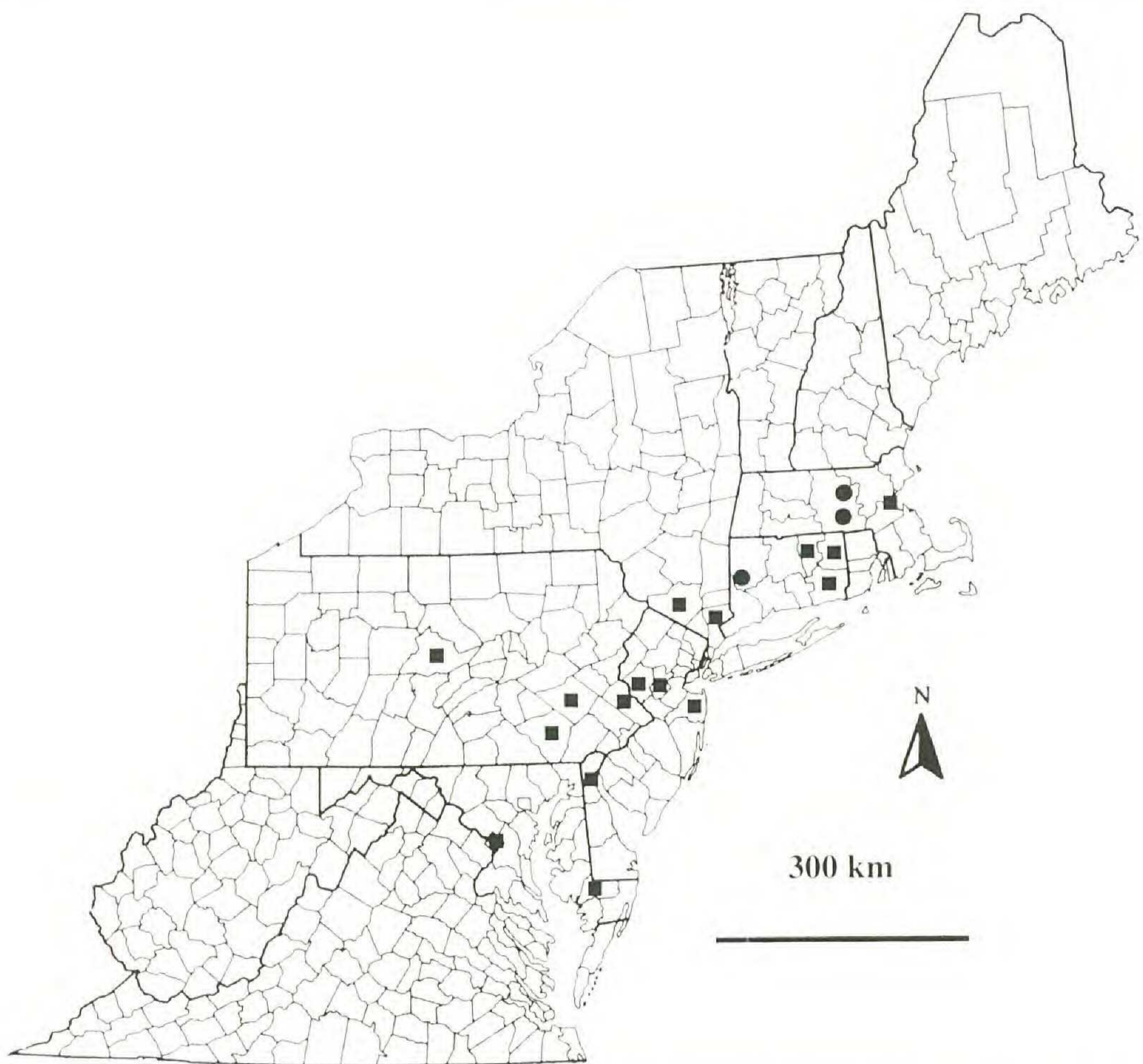


Figure 1. Historical distribution of *Desmodium humifusum* by county (■), and extant populations (●); redrawn from Rawinski 1990.

able information is insufficient to make a decision. *Desmodium humifusum* was placed in this category partly because Rawinski, in the Final Status Survey Report for the species, theorized that the plant could be a hybrid (Rawinski 1990). The Category 2 candidate list was discontinued by act of Congress on December 6, 1996 [Federal Register 61(235): 64481].

Desmodium humifusum was never common, based on a survey of herbarium specimens by Rawinski (1990) that yielded only 35 historic collections from four major herbaria (New York Botanical Garden, Gray Herbarium of Harvard University, New England Botanical Club, and Philadelphia Academy of Natural Sciences). These collections indicated a historical distribution roughly from Boston, Massachusetts to the District of Columbia, with 19 sites representing 16 counties in seven states and the District of Columbia (Figure 1). Although field surveys by Rawinski and others

Table 1. Morphological differences among *Desmodium paniculatum*, *D. humifusum*, and *D. rotundifolium*.

Trait/Species	<i>D. paniculatum</i>	<i>D. humifusum</i>	<i>D. rotundifolium</i>
Habit	Upright	Trailing	Prostrate
Stem pubescence	Glabrous or sparsely strigose	Sparsely long pilose and uncinulate	Densely long pilose and uncinulate
Stipules	Subulate and of- ten deciduous	Lanceolate and persistent	Broadly ovate and persistent
Leaflet shape	Lanceolate	Rhombic	Suborbicular

failed to re-locate this species at any of the historic locations, three new populations were discovered, two in Worcester County, Massachusetts, near Clinton and Oxford and a third near New Milford, Litchfield County, Connecticut (Figure 1). The Clinton population was estimated to contain 50–100 plants, whereas the other two populations had approximately 10 plants each. The general occurrence of *D. humifusum* in dense clusters of stems limited the precision of population estimates. Rawinski (1990) hypothesized a hybrid origin for *D. humifusum* involving *D. paniculatum* (L.) Alph. de Candolle and *D. rotundifolium* (Michx.) Alph. de Candolle based on morphological intermediacy and the invariable occurrence of the three species together.

Hybridization in *Desmodium* has been well-documented among several species used as forage crops in tropical climates (e.g., Chow 1982; Chow and Crowder 1972, 1973, 1974; Hutton and Gray 1967; Imrie and Blogg 1983; McWhirter 1969; Park and Rotar 1968; Rotar and Chow 1971; Rotar et al. 1967) and has been invoked to explain cases of intermediate morphologies among North American species of the genus (e.g., Isely 1953, 1983, 1990, 1998; Steyermark 1963; Vail 1892; Voss 1985). Furthermore, experimental crosses have demonstrated interfertility between several North American species [e.g., *D. viridiflorum* (L.) Alph. de Candolle \times *D. perplexum* B. G. Schub. and *D. laevigatum* (Nutt.) Alph. de Candolle \times *D. perplexum*; Raveill 1995] but no attempt has been made to cross *D. paniculatum* and *D. rotundifolium*.

The morphological differences between *Desmodium paniculatum* and *D. rotundifolium* are pronounced, with *D. humifusum* having roughly intermediate morphology (Table 1). The putative parental species are broadly sympatric with the entire range of *D.*

rotundifolium, from Massachusetts, Vermont, Michigan, and Kansas south to Florida and Texas (Great Plains Flora Association 1986) contained within the broader geographical range of *D. paniculatum*. However, the two species are generally separated ecologically. *Desmodium rotundifolium* is generally found in the interior of woodlands, while *D. paniculatum* occurs in more sunny habitats, including woodland openings and edges. The two species most often occur together when natural or man-made disturbance opens a woodland canopy and *D. paniculatum* moves into habitat previously occupied only by *D. rotundifolium* (Raveill, pers. obs.).

The proposed hybrid origin for *Desmodium humifusum* is supported by: 1) the close proximity of the three species at each location where *D. humifusum* occurs, 2) similar floral structure, 3) similar floral phenology, and 4) identical chromosome numbers. All three species are diploid with $2n = 22$ or $n = 11$ (Young 1940). The count for *D. humifusum* was reported for *D. glabellum* Michx. [= *Meibomia glabella* (Michx.) Kuntze] following the nomenclature at that time (Britton and Brown 1913; Robinson and Fernald 1908; Small 1933). Little variation in chromosome number has been found in *Desmodium*; all reported species have $2n = 22$, except for a few species from South America and Africa with $2n = 20$ (Rotar and Urata 1967; Turner and Fearing 1959). Polyploidy has never been reported in *Desmodium* or related genera (Ohashi et al. 1981).

Although morphological intermediacy is usually the initial criterion on which to base a hypothesis of hybrid origin, other explanations exist (Gottlieb 1972). Allozyme analysis can be used to test hypotheses of hybridization (Crawford 1990). The simple co-dominant inheritance of allozymes allows for the detection of additive profiles in hybrid taxa where parental taxa are fixed for different alleles or where allele frequencies differ significantly (Aparicio et al. 2000; Gallez and Gottlieb 1982; Hollingsworth et al. 1995; Johnson et al. 1998; Werth 1989). Although lack of differentiation between putative parental species can limit hypothesis testing, proposed parental species can sometimes be conclusively excluded (Harris and Abbott 1997).

In this study, allozyme analysis was used to test the null hypothesis that the three species were genetically discreet. The alternative hypothesis was a hybrid origin of *Desmodium humifus-*

um with *D. paniculatum* and *D. rotundifolium* as the putative parental species.

MATERIALS AND METHODS

Leaf tissue for protein extraction and electrophoresis was obtained from *Desmodium humifusum*, *D. paniculatum*, and *D. rotundifolium* plants at each of the three extant locations of *D. humifusum* (Figure 1). For comparison, a site in Lenawee County, Michigan, was chosen at which *D. paniculatum* and *D. rotundifolium* grew intermixed over an extensive area. At this site, neither *D. humifusum* nor any plants that seemed intermediate between *D. paniculatum* and *D. rotundifolium* occurred.

Sampling strategies varied because of the distribution of the species at each location. *Desmodium humifusum* occurred either as individual stems or in dense clusters of intertwined stems. Within clusters, determination of individuals was difficult. All isolated *D. humifusum* stems were sampled and several stems were sampled from each cluster of stems.

At the Clinton and Lenawee locations, plants of *Desmodium paniculatum* and *D. rotundifolium* were present throughout forested areas that had been heavily logged. Hundreds of plants of each species were present, with no apparent pattern to the fine-scale distribution of the two species. Sampling at these locations was confined to a roughly circular area of about 20 m in diameter.

The Oxford and New Milford locations were in powerline cuts, with sampling limited to these rights-of-way. The Oxford *Desmodium humifusum* population was about 20 m from a road and consisted of one cluster of about 10 stems and two isolated plants several meters away. Sampling of the other two species was done between the *D. humifusum* plants and the road. At the New Milford location, a single patch of about 50 stems of *D. humifusum* was present. The powerline right-of-way was heavily overgrown, with individuals of the other two species widely scattered; samples were obtained from an approximately 100 m length of the right-of-way.

The upper portion of each plant sampled was placed into an individual Zip-Lock[®] plastic bag and kept on ice during transport to Vanderbilt University, where all protein extractions and electrophoresis were performed. A voucher for each plant used in

electrophoresis was deposited at the herbarium of Central Missouri State University (WARM).

Horizontal starch gel electrophoresis followed procedures summarized in Wendel and Weeden (1989) and Werth (1985). Enzymes were extracted by hand-grinding approximately equal volumes of fresh leaf material and the simple buffer of Werth (1985) fortified with 10% (w/v) polyvinylpyrrolidone, average molecular weight 40,000, and 0.5% 2-mercaptoethanol. The crude extract was absorbed into wicks of Whatman No. 1 filter paper and inserted directly into 12% starch gels. Ten enzyme systems encoded 15 putative loci: aspartate aminotransferase (*Aat-1*, *Aat-2*), colorimetric esterase (*Est*), isocitrate dehydrogenase (*Idh-1*, *Idh-2*), leucine aminopeptidase (*Lap*), malate dehydrogenase (*Mdh-1*, *Mdh-2*), menadione reductase (*Mnr*), peroxidase (*Per*), phosphoglucomutase (*Pgm-1*, *Pgm-2*), 6-phosphogluconate dehydrogenase (*6-Pgd*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*). Visualization of enzymes followed Soltis et al. (1983), with the use of agar overlays and frozen premixed "zymecicles" (Werth 1990). Five buffer systems were used to resolve the loci:

1. lithium borate/tris citrate pH 8.3 (Soltis et al. 1983) resolved *Mnr* and *Tpi*;
2. tris citrate pH 8.0 (Werth 1985) resolved *Aat* and *Per*;
3. histidine-citrate pH 5.7 (Soltis et al. 1983) resolved *Est* and *Lap*;
4. tris maleate pH 7.4 (Werth 1985) resolved *Pgm* and *Mdh*;
5. morpholine citrate pH 8.0 (0.04 M citric acid titrated to pH 8.0 with n-3 aminopropyl morpholine), modified from Clayton and Tretiak (1972) was used to resolve *Idh* and *6-Pgd*.

All enzymes migrated anodally except *Per*, which migrated cathodally. Alleles were designated by letters, with the most anodally migrating allozyme denoted "a." Allele nomenclature was based on a more extensive study of *Desmodium*, with some alleles found in species or sites not reported here (Raveill 1995). The Mendelian inheritance of all variable loci has been reported for *D. paniculatum*, or for the related *D. perplexum*, using either controlled crosses or progeny arrays from single plants (Raveill 1995). No gene duplication was indicated, and all banding patterns and inheritance were consistent with the expectations of diploid species.

Allozyme data were used to determine various genetic attributes of each species and population. BIOSYS-1 (Swofford and Selander 1981) was used for all calculations except for the t-test of means, which followed Sokal and Rohlf (1981). Calculations for mean observed and mean expected heterozygosity per locus used direct counts and unbiased estimates, respectively. Wright's fixation index (F_{IS}) was used to express heterozygosity of individuals relative to the population in which they were found. Levene's correction for small sample size (Levene 1949) was employed in chi-square analysis. Allozyme similarity was assessed using Rogers similarity (Rogers 1972).

RESULTS

Seven of 15 loci were polymorphic in at least one of the putative parental species (Table 2). The only fixed difference discriminating these two species involved *Tpi-1*, at which *Desmodium paniculatum* contained alleles *b* or *c* while *D. rotundifolium* was fixed for allele *e*.

Genetic similarity obtained from pairwise comparisons of co-occurring *Desmodium paniculatum* and *D. rotundifolium* populations ranged from 0.705 at New Milford, Connecticut, to a maximum of 0.819 at the Oxford, Massachusetts site, with a mean of 0.797. The site at Lenawee County, Michigan, without *D. humifusum*, had a similarity of 0.800 indicating that the presence of *D. humifusum* did not cause the potential parental species to be genetically more similar.

All samples within each cluster of *Desmodium humifusum* stems consisted of a single allozyme genotype and was considered to represent a single clone. The actual number of genets of *D. humifusum* was far below previous estimates, being one, three, and four at New Milford, Oxford, and Clinton, respectively, for a total of eight genets known in 1992. Although some plants consisted of only a single stem, the largest clone, "Clinton-4," consisted of an estimated 100 stems over a roughly oval area of about 8 m².

No unique alleles were found in *Desmodium humifusum*; instead the alleles of *D. humifusum* were a composite of those of the putative parental species, *D. paniculatum* and *D. rotundifolium*. At the critical *Tpi-1* locus, seven of the eight *D. humifusum* individuals were heterozygous, combining the *e* allele of *D. ro-*

Table 2. Allele frequencies for polymorphic loci for populations of *Desmodium paniculatum* and *D. rotundifolium* from New Milford, Connecticut (NM); Oxford, Massachusetts (OX); Clinton, Massachusetts (CL); and Lenawee Co., Michigan (MI). Loci not listed were monomorphic for all sites. Mean allele frequencies for each species and sample sizes for each population are also included.

Locus	Allele	<i>D. paniculatum</i>					<i>D. rotundifolium</i>				
		NM	OX	CL	MI	Mean	NM	OX	CL	MI	Mean
<i>Aat-1</i>	<i>a</i>	0.0	0.0	0.0	0.167	0.042	0.0	0.0	0.0	0.0	0.0
	<i>b</i>	1.0	1.0	1.0	0.833	0.958	1.0	1.0	1.0	1.0	1.0
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66
<i>Est-1</i>	<i>a</i>	0.938	0.0	0.207	0.068	0.303	0.0	0.0	0.0	0.0	0.0
	<i>b</i>	0.063	0.882	0.638	0.932	0.629	0.0	0.0	0.0	0.0	0.0
	<i>c</i>	0.0	0.118	0.155	0.0	0.068	1.0	1.0	1.0	1.0	1.0
		N=8	N=17	N=28	N=37	N=90	N=11	N=7	N=6	N=17	N=41
<i>Idh-1</i>	<i>a</i>	1.0	0.086	1.0	0.676	0.690	1.0	1.0	0.275	1.0	0.819
	<i>b</i>	0.0	0.914	0.0	0.324	0.310	0.0	0.0	0.725	0.0	0.181
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66
<i>Idh-2</i>	<i>a</i>	1.0	1.0	0.333	0.865	0.800	1.0	1.0	1.0	1.0	1.0
	<i>b</i>	0.0	0.0	0.667	0.135	0.200	0.0	0.0	0.0	0.0	0.0
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66
<i>Pgm-1</i>	<i>a</i>	1.0	0.0	0.372	0.417	0.447	0.0	0.0	0.0	0.0	0.0
	<i>b</i>	0.0	1.0	0.628	0.583	0.553	1.0	1.0	0.950	1.0	0.988
	<i>c</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.050	0.0	0.012
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66

Table 2. Continued.

Locus	Allele	<i>D. paniculatum</i>					<i>D. rotundifolium</i>				
		NM	OX	CL	MI	Mean	NM	OX	CL	MI	Mean
<i>6-Pgd</i>	<i>a</i>	0.0	0.0	0.0	0.0	0.0	0.455	0.0	0.0	0.0	0.114
	<i>b</i>	1.0	1.0	1.0	1.0	1.0	0.545	1.0	1.0	1.0	0.886
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66
<i>Tpi-1</i>	<i>b</i>	1.0	0.897	1.0	1.0	0.974	0.0	0.0	0.0	0.0	0.0
	<i>c</i>	0.0	0.103	0.0	0.0	0.026	0.0	0.0	0.0	0.0	0.0
	<i>e</i>	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0
		N=8	N=29	N=43	N=37	N=117	N=11	N=18	N=20	N=17	N=66

Table 3. Allozymic genotypes of all individuals of *Desmodium humifusum* (e.g., MN-1, OX-1, etc.) for loci polymorphic in either *D. paniculatum* or *D. rotundifolium* at each site (Table 2). The number of stems examined for each clone is given.

Locus	Individual							
	NM-1 23	OX-1 4	OX-2 1	OX-3 1	CL-1 2	CL-2 3	CL-3 1	CL-4 6
<i>Aat-1</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>
<i>Est-1</i>	<i>bc</i>	<i>cc</i>	—	—	<i>bc</i>	<i>bc</i>	<i>cc</i>	<i>cc</i>
<i>Idh-1</i>	<i>ab</i>	<i>ab</i>	<i>bb</i>	<i>bb</i>	<i>ab</i>	<i>ab</i>	<i>ab</i>	<i>ab</i>
<i>Idh-2</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>	<i>aa</i>
<i>Pgm-1</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>ab</i>	<i>bb</i>	<i>bb</i>
<i>6-Pgd</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>
<i>Tpi-1</i>	<i>be</i>	<i>be</i>	<i>be</i>	<i>ce</i>	<i>be</i>	<i>be</i>	<i>bb</i>	<i>be</i>

tundifolium with either the *b* or *c* alleles of *D. paniculatum* (Table 3).

When compared with the two putative parental species, *Desmodium humifusum* had a significantly higher percentage of polymorphic loci ($p < 0.05$, t-test of means for planned comparisons) and mean number of alleles per locus ($p < 0.01$) than did *D. rotundifolium*, but was not significantly different from *D. paniculatum* for these measures (Table 4). Mean number of alleles per polymorphic locus did not differ between *D. humifusum* and either of the parental species. However, *D. humifusum* did have a significantly higher mean observed heterozygosity per locus and mean expected heterozygosity than either *D. paniculatum* or *D. rotundifolium* ($p < 0.001$, for each comparison). When the data for the Michigan population of the putative parental species were dropped from the calculations, because they could not directly contribute to the *D. humifusum* populations in New England, then *D. humifusum* had higher values for each measure of genetic variability than either putative parental species.

For both *Desmodium paniculatum* and *D. rotundifolium*, nearly every polymorphic locus showed a significant deficit of heterozygotes (Table 5). In sharp contrast, *D. humifusum* had an excess of heterozygotes at every polymorphic locus, although small sample sizes precluded calculations of statistical significance. The fixation index for the New Milford site was, by definition, -1.0 for all polymorphic loci (Table 5) since only one individual was present. Assuming that deviations were random, the chances of a

Table 4. Percentage of polymorphic loci, no criterion (P), mean number of alleles per polymorphic locus (A_p), mean number of alleles per locus (A), mean observed heterozygosity per locus (H_o), and mean expected heterozygosity for populations of *Desmodium paniculatum*, *D. rotundifolium*, and *D. humifusum*. Site abbreviations in Table 2.

Site	P	A_p	A	H_o	H_E
<i>D. paniculatum</i>					
NM	6.67	2.0	1.07	0.008	0.008
OX	20.00	2.0	1.20	0.015	0.038
CL	20.00	2.3	1.27	0.038	0.097
MI	33.33	2.0	1.33	0.056	0.106
Mean	20.00	2.08	1.22	0.029	0.062
<i>D. rotundifolium</i>					
NM	6.67	2.0	1.07	0.000	0.035
OX	0.00	—	1.00	0.000	0.000
CL	13.33	2.0	1.13	0.003	0.034
MI	0.00	—	1.00	0.000	0.000
Mean	5.00	2.0	1.05	0.001	0.017
<i>D. humifusum</i>					
NM	20.00	2.0	1.20	0.200	0.200
OX	13.33	2.5	1.20	0.089	0.071
CL	26.67	2.0	1.27	0.167	0.119
Mean	20.00	2.2	1.22	0.152	0.130

positive deviation were equal to those of a negative deviation at any given locus. Considering only populations with more than one plant, drawing six consecutive values that deviate in the same direction by chance is extremely unlikely ($p < 0.02$, sign test; Sokal and Rohlf 1981).

DISCUSSION

The alleles found in *Desmodium humifusum* are a subset of those in the other two species, which would be possible with three genetically isolated species. Neutral genetic polymorphisms may be shared among closely related species (Klein et al. 1998). Therefore, each of three diverged species could have independently received a portion of the allozyme variability of their most recent common ancestor. By chance, certain alleles might have been lost in both the *D. paniculatum* and *D. rotundifolium* lineages, but maintained in the lineage leading to *D. humifusum*.

Table 5. Wright's fixation index (F_{IS}) for all polymorphic loci from populations of *Desmodium paniculatum*, *D. rotundifolium*, and *D. humifusum*. Location abbreviations are given in Table 2. Chi-square test with Levene's correction for small samples was employed with values that statistically deviate from 0 ($p < 0.05$) indicated with an asterisk (*). Monomorphic loci in each population are indicated with a dash (—).

Locus	<i>D. paniculatum</i>				<i>D. rotundifolium</i>				<i>D. humifusum</i>		
	NM	OX	CL	MI	NM	OX	CL	MI	NM	OX	CL
<i>Aat-1</i>	—	—	—	1.000*	—	—	—	—	—	—	—
<i>Est-1</i>	-0.067	0.433*	0.803*	0.357*	—	—	—	—	-1.000	—	-0.333
<i>Idh-1</i>	—	0.781*	—	0.260	—	—	0.875*	—	-1.000	-0.200	-1.000
<i>Idh-2</i>	—	—	0.679*	0.306*	—	—	—	—	—	—	—
<i>Pgm-1</i>	—	—	0.303*	0.429*	—	—	1.000*	—	—	—	-0.143
<i>6-Pgd</i>	—	—	—	—	1.000*	—	—	—	—	—	—
<i>Tpi-1</i>	—	0.628*	—	—	—	—	—	—	-1.000	-0.636	-0.600

While this possibility cannot be excluded, it seems unlikely and could not be easily tested.

However, the high level of heterozygosity in *Desmodium humifusum* would be difficult to explain if it were a lineage perpetuated by sexual reproduction. The ratio of observed to expected heterozygosity in *D. humifusum* exceeds that of the putative parental species and even that of a panmictic population. One generation of sexual reproduction would reduce the level of heterozygosity to that predicted by Hardy-Weinberg.

It would be surprising for an exceedingly rare species, such as *Desmodium humifusum*, to be as genetically diverse as its common and geographically widespread congeners. Geographically widespread species generally have higher levels of genetic diversity than species with restricted distributions (Baskauf et al. 1994; Karron 1991; Rieseberg et al. 1989). A loss of genetic diversity would be expected in *D. humifusum* because of its occurrence as a limited number of scattered populations, all of which have extremely small population sizes (Ellstrand and Elam 1993).

Clearly, the alternative hypothesis of hybridization is a more parsimonious explanation of the allozyme data, as this would explain both the high heterozygosity and the composite nature of the alleles of *Desmodium humifusum*. The excessive heterozygosity of *D. humifusum* was expected since the possible parental species were genetically differentiated. The most informative locus for assessing hybridization was *Tpi-1* because of fixed differences between the possible parental species. All individuals of *D. humifusum* except one were heterozygous at this locus, combining alleles unique to the parental taxa.

The *Tpi-1^c* allele is of interest because it was not encountered elsewhere in a rangewide survey of *Desmodium paniculatum* (Raveill 1995). Because this allele occurred at the Oxford location in both *D. paniculatum* and in one of the three individuals of *D. humifusum*, observations support local hybridization, rather than long-distance dispersal as the source of this hybrid.

However genotypes of half of the *Desmodium humifusum* plants did not match the composites expected of F₁ hybrids based on the alleles of the parental species at each site. Three examples, the “Clinton-3” plant, homozygous at the *Tpi-1* locus, and “Oxford-2” and “Oxford-3” plants, homozygous at the *Idh-1* locus, could be explained if they were sired either by selfing or backcrossing to *D. paniculatum*.

The single *Desmodium humifusum* plant at New Milford did not match the expected composite profile at two loci, *Pgm-1* and *Idh-1*. The homozygous *Pgm-1* locus can be explained by selfing or backcrossing with *D. rotundifolium* but the *Idh-1* locus is more difficult to explain. The *D. humifusum* plant was heterozygous even though both parental species were fixed for the same allele. Hypothetically, the “missing” *Idh-1^b* allele could have come from either parental species, since both species contained this allele at other locations. Several hypotheses could be advanced, including dispersal from a distant location, inadequate sampling, or loss of alleles in the parental species. Details of the New Milford location tend to support one of the latter two. Much of the powerline cut was heavily overgrown with young trees, making it difficult to locate *Desmodium* plants. While all individuals of the parental species encountered were sampled, additional plants could have been missed. Also the dense woody growth greatly reduced available habitat for all herbaceous species, including *Desmodium*. Alleles may have been lost as the populations decreased.

In an early and insightful discussion of hybridization, Wiegand (1935) commented that “. . . hybrids seem like swarms of bees, buzzing around for a time, only to disappear, leaving the fundamental species to continue through the ages.” Such may be the case with *Desmodium humifusum*; however several traits—such as fertility, perennial habit, and clonal growth—increase the potential for hybridization to have a more profound evolutionary role (Arnold 1997; Burke et al. 2000). The present study provides limited information relevant to the evolutionarily consequences of hybridization, such as introgression or diploid speciation. Introgression may be absent or if it is occurring, then the level of gene flow between the parental species must be low, based on allele frequency differences at several loci. Also genetic similarities between parental species were no greater at sites where *D. humifusum* was present than at the site where the hybrid was absent. However, the failure to detect introgression at a few allozyme loci is not conclusive evidence against introgression (Rieseberg and Wendel 1993).

Because of its hybrid status, *Desmodium humifusum* cannot receive federal listing. The endangered species act has no provision for the listing of hybrids between species that are not themselves rare, even if the hybrid is extremely sporadic in its occurrence [Federal Register 61(26): 4710]. This public policy fails to

recognize the uniqueness of sites of rare hybridization events and their potential scientific significance (Whitham et al. 1991). Hybridization and subsequent backcrossing with the parental species can form a genetic bridge between species (Arnold 1994). The unique gene combinations created have the potential of allowing for the exploitation of habitat not suitable to either of the parental species (Cade 1983) and, thus, may be especially important in an evolutionary context (Levin 1970; Stace 1987).

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